

CLAIMS

1. A protein (C) or (D):

(C) a protein having an amino acid sequence described in SEQ ID NO: 15 of the Sequence Listing,

5 (D) a protein consisting of an amino acid sequence that includes substitution, deletion, insertion, addition or inversion of one or a plurality of amino acids in the amino acid sequence described in SEQ ID NO: 15 of the Sequence Listing, and has activity to produce a dipeptide from an L-amino acid ester and an L-amino acid.

10

2. A protein (E) or (F):

(E) a protein having an amino acid sequence described in SEQ ID NO: 17 of the Sequence Listing,

(F) a protein consisting of an amino acid sequence that includes
15 substitution, deletion, insertion, addition or inversion of one or a plurality of amino acids in the amino acid sequence described in SEQ ID NO: 17 of the Sequence Listing, and has activity to produce a dipeptide from an L-amino acid ester and an L-amino acid.

20 3. A DNA (c) or (d):

(c) a DNA consisting of a base sequence consisting of bases numbers 486 to 1496 in a base sequence described in SEQ ID NO: 14 of the Sequence Listing,

(d) a DNA that hybridizes under stringent conditions with a DNA
25 consisting of a base sequence complementary to a base sequence

consisting of bases numbers 486 to 1496 in the base sequence described in SEQ ID NO: 14 of the Sequence Listing, and encodes a protein having activity to form a dipeptide from an L-amino acid ester and an L-amino acid.

5

4. A DNA (e) or (f):

(e) a DNA consisting of a base sequence consisting of bases numbers 311 to 1279 in a base sequence described in SEQ ID NO: 16 of the Sequence Listing,

10 (f) a DNA that hybridizes under stringent conditions with a DNA consisting of a base sequence complementary to a base sequence consisting of bases numbers 311 to 1279 in the base sequence described in SEQ ID NO: 16 of the Sequence Listing, and encodes a protein having activity to form a dipeptide from an L-amino acid ester
15 and an L-amino acid.

5. The DNA according to claim 3, wherein the stringent conditions are conditions under which washing is carried out at 60°C and at a salt concentration equivalent to 1×SSC and 0.1% SDS.

20

6. The DNA according to claim 4, wherein the stringent conditions are conditions under which washing is carried out at 60°C and at a salt concentration equivalent to 1×SSC and 0.1% SDS.

25 7. A recombinant DNA comprising incorporated therein the DNA

according to claim 3.

8. A recombinant DNA comprising incorporated therein the DNA according to claim 4.

5

9. A recombinant DNA comprising incorporated therein the DNA according to claim 5.

10. A recombinant DNA comprising incorporated therein the DNA according to claim 6.

11. A transformed cell comprising incorporated therein the DNA according to claim 3 in a state where the DNA is able to express a protein encoded thereby.

15

12. A transformed cell comprising incorporated therein the DNA according to claim 4 in a state where the DNA is able to express a protein encoded thereby.

20 13. A transformed cell comprising incorporated therein the DNA according to claim 5 in a state where the DNA is able to express a protein encoded thereby.

14 A transformed cell comprising incorporated therein the DNA
25 according to claim 6 in a state where the DNA is able to express a

protein encoded thereby.

15. A method for producing a dipeptide-forming enzyme, comprising:
culturing the transformed cells according to claim 11 in a medium, and
5 accumulating a protein having activity to produce the dipeptide from an
L-amino acid ester and an L-amino acid in the medium and/or in the
transformed cells.

16. A method for producing a dipeptide-forming enzyme, comprising:
10 culturing the transformed cells according to claim 12 in a medium, and
accumulating a protein having activity to produce the dipeptide from an
L-amino acid ester and an L-amino acid in the medium and/or in the
transformed cells.

15 17. A method for producing a dipeptide-forming enzyme, comprising:
culturing the transformed cells according to claim 13 in a medium, and
accumulating a protein having activity to produce the dipeptide from an
L-amino acid ester and an L-amino acid in the medium and/or in the
transformed cells.

20

18. A method for producing a dipeptide-forming enzyme, comprising:
culturing the transformed cells according to claim 14 in a medium, and
accumulating a protein having activity to produce the dipeptide from an
L-amino acid ester and an L-amino acid in the medium and/or in the
25 transformed cells.

19. A method for producing a dipeptide, comprising: producing a dipeptide from an L-amino acid ester and an L-amino acid using a protein having activity to form the dipeptide from an L-amino acid ester
5 and an L-amino acid that is produced in the transformed cells according to claim 11.

20. A method for producing a dipeptide, comprising: producing a dipeptide from an L-amino acid ester and an L-amino acid using a
10 protein having activity to form the dipeptide from an L-amino acid ester and an L-amino acid that is produced in the transformed cells according to claim 12.

21. A method for producing a dipeptide, comprising: producing a
15 dipeptide from an L-amino acid ester and an L-amino acid using a protein having activity to form the dipeptide from an L-amino acid ester and an L-amino acid that is produced in the transformed cells according to claim 13.

20 22. A method for producing a dipeptide, comprising: producing a dipeptide from an L-amino acid ester and an L-amino acid using a protein having activity to form the dipeptide from an L-amino acid ester and an L-amino acid that is produced in the transformed cells according to claim 14.

23. The method for producing a dipeptide according to claim 19,
wherein the L-amino acid ester is one or more types selected from the
group consisting of an L-alanine ester, a glycine ester, an L-valine ester,
5 an L-isoleucine ester, an L-methionine ester, an L-phenylalanine ester,
an L-serine ester, an L-threonine ester, an L-glutamine ester, an
L-tyrosine ester, an L-arginine ester, an L-aspartic acid- α -ester, an
L-aspartic acid- β -ester, an L-leucine ester, an L-asparagine ester, an
L-lysine ester, an L-aspartic- α,β -dimethyl ester and an
10 L-glutamine- γ -ester.

24. The method for producing a dipeptide according to claim 20,
wherein the L-amino acid ester is one or more types selected from the
group consisting of an L-alanine ester, a glycine ester, an L-valine ester,
15 an L-isoleucine ester, an L-methionine ester, an L-phenylalanine ester,
an L-serine ester, an L-threonine ester, an L-glutamine ester, an
L-tyrosine ester, an L-arginine ester, an L-aspartic acid- α -ester, an
L-aspartic acid- β -ester, an L-leucine ester, an L-asparagine ester, an
L-lysine ester, an L-aspartic- α,β -dimethyl ester and an
20 L-glutamine- γ -ester.

25. The method for producing a dipeptide according to claim 21,
wherein the L-amino acid ester is one or more types selected from the
group consisting of an L-alanine ester, a glycine ester, an L-valine ester,
25 an L-isoleucine ester, an L-methionine ester, an L-phenylalanine ester,

an L-serine ester, an L-threonine ester, an L-glutamine ester, an
 L-tyrosine ester, an L-arginine ester, an L-aspartic acid- α -ester, an
 L-aspartic acid- β -ester, an L-leucine ester, an L-asparagine ester, an
 L-lysine ester, an L-aspartic- α,β -dimethyl ester and an
 5 L-glutamine- γ -ester.

26. The method for producing a dipeptide according to claim 22,
 wherein the L-amino acid ester is one or more types selected from the
 group consisting of an L-alanine ester, a glycine ester, an L-valine ester,
 10 an L-isoleucine ester, an L-methionine ester, an L-phenylalanine ester,
 an L-serine ester, an L-threonine ester, an L-glutamine ester, an
 L-tyrosine ester, an L-arginine ester, an L-aspartic acid- α -ester, an
 L-aspartic acid- β -ester, an L-leucine ester, an L-asparagine ester, an
 L-lysine ester, an L-aspartic- α,β -dimethyl ester and an
 15 L-glutamine- γ -ester.

27. The method for producing a dipeptide according to claim 19,
 wherein the L-amino acid is one or more types selected from the group
 consisting of L-glutamine, L-asparagine, glycine, L-alanine, L-leucine,
 20 L-methionine, L-proline, L-phenylalanine, L-tryptophan, L-serine,
 L-threonine, L-tyrosine, L-lysine, L-arginine, L-histidine and
 L-glutamate.

28. The method for producing a dipeptide according to claim 20,
 25 wherein the L-amino acid is one or more types selected from the group

consisting of L-glutamine, L-asparagine, glycine, L-alanine, L-leucine, L-methionine, L-proline, L-phenylalanine, L-tryptophan, L-serine, L-threonine, L-tyrosine, L-lysine, L-arginine, L-histidine and L-glutamate.

5

29. The method for producing a dipeptide according to claim 21, wherein the L-amino acid is one or more types selected from the group consisting of L-glutamine, L-asparagine, glycine, L-alanine, L-leucine, L-methionine, L-proline, L-phenylalanine, L-tryptophan, L-serine,
10 L-threonine, L-tyrosine, L-lysine, L-arginine, L-histidine and L-glutamate.

30. The method for producing a dipeptide according to claim 22, wherein the L-amino acid is one or more types selected from the group
15 consisting of L-glutamine, L-asparagine, glycine, L-alanine, L-leucine, L-methionine, L-proline, L-phenylalanine, L-tryptophan, L-serine, L-threonine, L-tyrosine, L-lysine, L-arginine, L-histidine and L-glutamate.

20 31. The method for producing a dipeptide according to claim 23, wherein the L-amino acid is one or more types selected from the group consisting of L-glutamine, L-asparagine, glycine, L-alanine, L-leucine, L-methionine, L-proline, L-phenylalanine, L-tryptophan, L-serine, L-threonine, L-tyrosine, L-lysine, L-arginine, L-histidine and
25 L-glutamate.

32. The method for producing a dipeptide according to claim 24,
wherein the L-amino acid is one or more types selected from the group
consisting of L-glutamine, L-asparagine, glycine, L-alanine, L-leucine,
5 L-methionine, L-proline, L-phenylalanine, L-tryptophan, L-serine,
L-threonine, L-tyrosine, L-lysine, L-arginine, L-histidine and
L-glutamate.

33. The method for producing a dipeptide according to claim 25,
10 wherein the L-amino acid is one or more types selected from the group
consisting of L-glutamine, L-asparagine, glycine, L-alanine, L-leucine,
L-methionine, L-proline, L-phenylalanine, L-tryptophan, L-serine,
L-threonine, L-tyrosine, L-lysine, L-arginine, L-histidine and
L-glutamate.

15

34. The method for producing a dipeptide according to claim 26,
wherein the L-amino acid is one or more types selected from the group
consisting of L-glutamine, L-asparagine, glycine, L-alanine, L-leucine,
L-methionine, L-proline, L-phenylalanine, L-tryptophan, L-serine,
20 L-threonine, L-tyrosine, L-lysine, L-arginine, L-histidine and
L-glutamate.

35. A method for producing a dipeptide comprising: allowing a
protein having proline iminopeptidase activity to act on an L-amino acid
25 ester and an L-amino acid to form the dipeptide.

36 The method for producing a dipeptide according to claim 35,
wherein the protein having proline iminopeptidase activity is derived
from a microbe belonging to genus *Corynebacterium*, *Pseudomonas* or
5 *Bacillus*.

37 The method for producing a dipeptide according to claim 35,
wherein the protein having proline iminopeptidase activity is derived
from any of *Corynebacterium glutamicum*, *Pseudomonas putida* and
10 *Bacillus coagulans*.

38. The method for producing a dipeptide according to claim 35
wherein the L-amino acid ester is one or more types selected from the
group consisting of an L-alanine ester, a glycine ester, an L-valine ester,
15 an L-isoleucine ester, an L-methionine ester, an L-phenylalanine ester,
an L-serine ester, an L-threonine ester, an L-glutamine ester, an
L-tyrosine ester, an L-arginine ester, an L-aspartic acid- α -ester, an
L-aspartic acid- β -ester, an L-leucine ester, an L-asparagine ester, an
L-lysine ester, an L-aspartic- α,β -dimethyl ester and an
20 L-glutamine- γ -ester.

39. The method for producing a dipeptide according to claim 36
wherein the L-amino acid ester is one or more types selected from the
group consisting of an L-alanine ester, a glycine ester, an L-valine ester,
25 an L-isoleucine ester, an L-methionine ester, an L-phenylalanine ester,

an L-serine ester, an L-threonine ester, an L-glutamine ester, an L-tyrosine ester, an L-arginine ester, an L-aspartic acid- α -ester, an L-aspartic acid- β -ester, an L-leucine ester, an L-asparagine ester, an L-lysine ester, an L-aspartic- α,β -dimethyl ester and an

5 L-glutamine- γ -ester.

40. The method for producing a dipeptide according to claim 37 wherein the L-amino acid ester is one or more types selected from the group consisting of an L-alanine ester, a glycine ester, an L-valine ester,
10 an L-isoleucine ester, an L-methionine ester, an L-phenylalanine ester, an L-serine ester, an L-threonine ester, an L-glutamine ester, an L-tyrosine ester, an L-arginine ester, an L-aspartic acid- α -ester, an L-aspartic acid- β -ester, an L-leucine ester, an L-asparagine ester, an L-lysine ester, an L-aspartic- α,β -dimethyl ester and an

15 L-glutamine- γ -ester.

41 The method for producing a dipeptide according to claim 35, wherein the L-amino acid is one or more types selected from the group consisting of L-glutamine, L-asparagine, glycine, L-alanine, L-leucine,
20 L-methionine, L-proline, L-phenylalanine, L-tryptophan, L-serine, L-threonine, L-tyrosine, L-lysine, L-arginine, L-histidine and L-glutamate.

42 The method for producing a dipeptide according to claim 36,
25 wherein the L-amino acid is one or more types selected from the group

consisting of L-glutamine, L-asparagine, glycine, L-alanine, L-leucine, L-methionine, L-proline, L-phenylalanine, L-tryptophan, L-serine, L-threonine, L-tyrosine, L-lysine, L-arginine, L-histidine and L-glutamate.

5

43 The method for producing a dipeptide according to claim 37, wherein the L-amino acid is one or more types selected from the group consisting of L-glutamine, L-asparagine, glycine, L-alanine, L-leucine, L-methionine, L-proline, L-phenylalanine, L-tryptophan, L-serine,
10 L-threonine, L-tyrosine, L-lysine, L-arginine, L-histidine and L-glutamate.

44 The method for producing a dipeptide according to claim 38, wherein the L-amino acid is one or more types selected from the group
15 consisting of L-glutamine, L-asparagine, glycine, L-alanine, L-leucine, L-methionine, L-proline, L-phenylalanine, L-tryptophan, L-serine, L-threonine, L-tyrosine, L-lysine, L-arginine, L-histidine and L-glutamate.

20 45 The method for producing a dipeptide according to claim 39, wherein the L-amino acid is one or more types selected from the group consisting of L-glutamine, L-asparagine, glycine, L-alanine, L-leucine, L-methionine, L-proline, L-phenylalanine, L-tryptophan, L-serine, L-threonine, L-tyrosine, L-lysine, L-arginine, L-histidine and
25 L-glutamate.

46 The method for producing a dipeptide according to claim 40,
wherein the L-amino acid is one or more types selected from the group
consisting of L-glutamine, L-asparagine, glycine, L-alanine, L-leucine,
5 L-methionine, L-proline, L-phenylalanine, L-tryptophan, L-serine,
L-threonine, L-tyrosine, L-lysine, L-arginine, L-histidine and
L-glutamate.

47. A method for producing a dipeptide, comprising: producing the
10 dipeptide from an amino acid ester and an amino acid using a culture of
a microbe belonging to the genus *Corynebacterium*, *Pseudomonas* or
Bacillus and having the ability to produce the dipeptide from the amino
acid ester and the amino acid, microbial cells isolated from the culture
or a treated microbial product of the microbe.

15

48 The method for producing a dipeptide according to claim 47,
wherein the L-amino acid ester is one or more types selected from the
group consisting of an L-alanine ester, a glycine ester, an L-valine ester,
an L-isoleucine ester, an L-methionine ester, an L-phenylalanine ester,
20 an L-serine ester, an L-threonine ester, an L-glutamine ester, an
L-tyrosine ester, an L-arginine ester, an L-aspartic acid- α -ester, an
L-aspartic acid- β -ester, an L-leucine ester, an L-asparagine ester, an
L-lysine ester, an L-aspartic- α,β -dimethyl ester and an
L-glutamine- γ -ester.

25

- 49 The method for producing a dipeptide according to claim 47,
wherein the L-amino acid is one or more types selected from the group
consisting of L-glutamine, L-asparagine, glycine, L-alanine, L-leucine,
L-methionine, L-proline, L-phenylalanine, L-tryptophan, L-serine,
5 L-threonine, L-tyrosine, L-lysine, L-arginine, L-histidine and
L-glutamate.